

Dietary Boron as a Physiological Regulator of the Normal Inflammatory Response: A Review and Current Research Progress

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A substantial number of metabolic processes in humans and animals are beneficially affected by physiologic amounts of dietary boron. There is emerging evidence that boron influences immune function. Specifically, there is evidence that dietary boron helps control the normal inflammatory process and may do so by serving as a signal suppressor that down-regulates specific enzymatic activities typically elevated during inflammation at the inflammation site. Suppression, but not elimination, of these enzyme activities by boron is hypothesized to reduce the incidence and severity of inflammatory disease. This is a review of previous findings describing an apparent positive effect of boron on aspects of physiology related to the inflammatory process, including joint swelling, restricted movement, fever, antibody production, hemostasis, serine protease and lipoxygenase activities, and leukotriene metabolism. It also summarizes current research findings on the immunomodulatory effects of physiologic amounts of dietary boron such as reduced paw swelling and circulating neutrophil concentrations and increased circulating concentrations of natural killer cells and CD8a⁺/CD4⁺ cells of rats with antigen-induced arthritis. Possible biochemical mechanisms for the effects of boron on the induced inflammatory response are discussed, with emphasis on possible roles of boron in the respiratory burst mechanism, and inhibition of leukocyte 6-phosphogluconate dehydrogenase, gamma-glutamyl transpeptidase, cyclooxygenase, and serine proteases (elastase, chymase, cathepsin G, thrombin, and coagulation factors IXa, Xa, XIa) activities. *J. Trace Elem. Exp. Med.* 12:221–233, 1999.

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INTRODUCTION

A substantial number of metabolic processes in humans and animals are beneficially influenced by physiologic amounts of dietary boron [1]. There is emerging

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evidence that boron supports immune function. Specifically, dietary boron apparently acts to regulate the normal inflammatory process by serving as a signal suppressor that down-regulates the activities of specific enzymes involved in the inflammatory process and thus may play a role in modulating development of inflammatory disease. Suppression, but not elimination, of activities of these enzyme reduces the incidence and severity of the symptoms of inflammatory disease. Here, we review the literature describing the effect of boron on the immune system and the inflammatory process and summarize current research findings on the immunomodulatory effects of physiologic amounts of dietary boron.

INFLAMMATION AND INFLAMMATORY DISEASE

The normal inflammatory response serves to focus host defenses at a site of tissue injury or infection [2]. Thus inflammation is a localized protective response elicited by injury or destruction of tissues, which serves to destroy, dilute, or wall-off both the injurious agent and the injured tissue. In most instances, elimination of antigens proceeds without evidence of clinically detectable inflammation and, in fact, absence of inflammation after introduction of an injurious agent leads to a compromised host [3]. Excessive inflammation, either secondary to abnormal recognition of host tissue as "foreign" or failure to halt an otherwise normal inflammatory process, leads to inflammatory disease. In some inflammatory diseases, i.e., rheumatoid arthritis, the inciting agent is unknown or may be related to normal host tissue components [3].

BORON AND INFLAMMATORY DISEASE

The Unani traditional medical system in India uses sodium tetraborate or borax as an ingredient of some prescriptions for treatment of inflammatory diseases including joint pain [4]. In the only reported controlled human study for examination of dietary boron and inflammation interactions [5], 20 patients presenting radiographically confirmed osteoarthritis received either daily 6 mg (0.55 mmol) of boron (as sodium tetraborate decahydrate [borax]) as oral supplements or a placebo for 8 weeks in a double-blind trial. The arthritic individuals who received boron supplements self-reported substantial improvement in subjective measures of their arthritic condition (joint swelling, restricted movement). Rheumatoid factor, not measured in this arthritic study, is rapidly and completely precipitated in boric acid solutions (2%) in vitro [6]. This observation prompted the hypothesis that boron reacts with sugar moieties in the rheumatoid factor to form a reversible complex [6]. Formation of reversible complexes between boron and ligands that contain a ribosyl moiety (that contain adjacent *cis*-hydroxy groups) is a well-studied phenomenon [1].

Although initial findings were probably based on the pharmacologic actions of boron, animal models of rheumatoid arthritis seem to respond positively to boron. For example, boron (10 mg/kg body weight) as common borax was reported to have antiarthritic and antipyretic activities because it reduced paw volume and fever in albino rats with formaldehyde-induced arthritis [4]. A recent preliminary report from our group [7] suggested that ample (but probably not pharmacologic) amounts of dietary boron (20 μ g [1.85 μ mol]/g) compared to very low amounts (<0.2 μ g [0.02

$\mu\text{mol/g}$), significantly delayed the onset of adjuvant-induced arthritis in vitamin D adequate rats (incidence of arthritis at 12 d postinjection with *M. tuberculosis* [expressed as % of animals in each dietary treatment]: $<0.2 \mu\text{g B/g}$, 41%; $20 \mu\text{g B/g}$, 0%). The concentration of boron in commercial rodent chow is typically $\sim 13.0 \text{ mg/kg}$ [8]. In a separate study, addition of boron in vitro over a range between 0 and $20 \mu\text{g}$ [$1.85 \mu\text{mol/mL}$] inhibited proliferation of splenic cells isolated from boron-deprived rats and subsequently stimulated by 0, 5, or $50 \mu\text{g}$ phytohemagglutinin/mL [7]. A study from our laboratory with physiologic amounts of boron ($3 \mu\text{g}$ [$0.28 \mu\text{mol/g}$]) added to a boron-low diet ($0.2 \mu\text{g}$ [$0.02 \mu\text{mol/g}$]) revealed that supplemental boron more than doubled serum total antibody concentrations to injected antigen (human typhoid vaccine) in vitamin D adequate rats [9].

BORON AND THE RESPIRATORY BURST MECHANISM

Chemoattractants that are produced and released at the inflammation site direct the migration of polymorphonuclear (PMN) leukocytes to the site. Failure to end PMN leukocyte recruitment creates a counterproductive cycle in which activated PMN leukocytes release the very potent chemoattractant, LTB_4 , that attracts other PMN leukocytes [10]. Thus more than one billion PMN leukocytes, mainly neutrophils, may be drawn into the joint cavity of the knee each day in a rheumatoid patient with moderately active disease [10]. Activated neutrophils and other phagocytes produce large quantities of superoxide, the precursor of a group of powerful reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), hydroperoxy radical (HO_2^\cdot), and the hydroxyl radical (OH^\cdot) that are used as microbicidal agents during the normal inflammatory process. The ROS are released into a phagocytic vacuole or the extracellular space.

The process of ROS generation causes phagocytes to consume much more oxygen than that needed for the generation of energy required for phagocytosis. The phenomenon is termed respiratory burst, a poor name because it is unrelated to mitochondrial electron transport. The primary electron donor for the reduction of oxygen during respiratory burst is nicotinamide-adenine dinucleotide phosphate (NADPH) [11].



NADPH oxidase.

The source of the NADPH for the respiratory burst comes mainly from the reduction of NADP^+ in the pentose-phosphate (P-P) pathway, which is very active during the respiratory burst. In plants, one substrate of the P-P pathway, 6-phosphogluconate, is known to complex with boron, which thereby inhibits 6-phosphogluconate dehydrogenase (PGD), a key enzyme in the P-P pathway [12]. Thus a serious problem in boron-deficient plants is an increase in the amount of substrate metabolized via the

P-P pathway, which gives rise to overabundant synthesis of phenolic compounds and subsequent death of plants in the subclass *Dicotyledoneae* [12]. It is reasonable to hypothesize that proper boron nutriture causes a simple reduction in leukocyte ROS generation through down-regulation of leukocyte PGD with subsequent alleviation of the arthritic symptoms. Vigorous attempts are being made in this laboratory to directly measure leukocyte respiratory burst activity in joint cavities during boron deprivation.

BORON AND ROS METABOLISM

There is emerging evidence that boron also hastens destruction of ROS that are scavenged and destroyed by defense mechanisms that employ glutathione (GSH), superoxide dismutase (SOD), and catalase.

GSH. Glutathione peroxidase reduces hydrogen peroxide by means of reduced GSH, and the intracellular reduction of GSH requires NADPH and glutathione reductase [13]. Gamma-glutamyl transpeptidase (GGT) is the major catabolic enzyme for GSH and its derivatives. Serine-borate complex is a transition-state inhibitor of GGT [14] and by that mechanism, apparently elevates the concentrations of GSH in cultured fibroblasts taken from individuals suffering from GSH synthase deficiency [15]. In rats, serine-borate was reported to increase renal GSH content in vivo [16]. In the only controlled human study of boron and inflammation described above [5], patients consuming the boron supplement (6 mg [0.55 mmol]/d) exhibited lower blood GGT concentrations (20.7 U [0.345 μ kat]/L vs. 26.3 U [0.438 μ kat]/L) and also reported substantial improvement in subjective measures of their arthritic condition (pain on movement, joint swelling, restricted movement). Efforts are underway in this laboratory to confirm the corollary of the boron-inflammation hypothesis that boron limits oxidative damage by enhancing body stores of glutathione and its derivatives or inducing other ROS neutralizing agents.

SOD and catalase. SOD is an oxidoreductase that serves to dismutate superoxide anions that are generated during oxidative metabolism and in response to noxious stimuli. Catalase disproportionates hydrogen peroxide and protects membrane lipids and proteins from attack by peroxy radicals [17]. Boron supplementation significantly increased erythrocyte SOD activity ($2,578 \pm 74$ vs. $2,257 \pm 99$ U/g hemoglobin; $P < 0.03$) in men and postmenopausal women with marginal copper status [18]. It remains to be determined whether SOD activity increased because boron induced free radical formation, or whether boron improved antioxidative capacity. In rats, physiologic amounts of dietary boron decreased RBC catalase activity [19]. However, boron-deficient *Anabaena* PCC 7119 heterocysts exhibit increased concentrations of SOD, catalase, and peroxidase, a situation thought to arise as a consequence of alterations in the heterocyst envelope with subsequent facilitated O_2 diffusion and an increase in ROS [20].

BORON AND SERINE PROTEASES

There is emerging evidence that boron facilitates the normal inflammatory process by dampening the activity of the serine proteases. During a severe inflammatory response, various blood and tissue cells, including PMNs, release lysosomal proteinases both extracellularly and into the circulation. These enzymes, as well as the

liberated ROS, exacerbate the inflammatory response by degrading connective tissue structures, membrane constituents, and soluble proteins by proteolysis [21]. Uncontrolled enzymatic degradation of tissue is normally prevented by the proteinase inhibitors, a family of soluble proteins (antiproteases) that bind and inactivate the proteolytic enzymes [22]. Compromised status of these protease inhibitors can lead to a myriad of diseases, including rheumatoid arthritis, which is essentially a problem of abnormal connective tissue turnover because of uncontrolled proteolysis [23–25].

The serine proteases (E.C. 3.4.21) are a sub-subclass of hydrolases and are major proteolytic enzymes (i.e., elastase, chymase, and cathepsin G) released by activated leukocytes that, in addition to degrading structural proteins, have many essential regulatory roles in normal inflammation, including control of the blood complement system (e.g., complement Factor I), the contact activation system (e.g., tissue kallikrein), the fibrinolytic system (e.g., thrombin), and the coagulation system (e.g., coagulation Factor Xa) [26]. The inactive (zymogenic) forms of the serine proteases are stored intracellularly or circulate extracellularly and require proteolytic cleavage for functional activity. The activated enzymes are characterized by a catalytic triad of invariant amino acid residues (histidine 57, aspartic acid 102, serine 195), which form a “charge relay” system essential for their catalytic activity [27, 28]. During the course of all serine protease-catalyzed amide and ester hydrolyses, the SerOH group becomes transiently acylated [29]. Boron compounds reversibly inhibit the activity of many serine proteases. For example, a synthetic boronic acid was a highly effective, reversible inhibitor of elastase (1:1 complex at 23 μ M), which reduced experimental emphysema in hamsters induced by intratracheally administered elastase [30]. Nanomolar concentrations of certain synthetic peptide boronic acids, including MeO-Suc-Ala-Ala-Pro-acetamido-2-phenylethane boronic acid, effectively inhibit chymotrypsin, cathepsin G, and both leukocyte and pancreatic elastase in vitro [31].

Boron compounds are thought to reversibly inhibit the activity of serine proteases when the boron atom forms a tetrahedral boron adduct that mimics the tetrahedral adduct formed during normal substrate hydrolysis [32–34]. This phenomenon has been studied with substituted boric acid compounds (e.g., arylboronic acids). The available experimental evidence [35] suggests that formation of the boron adduct occurs when boron completes its octet by reacting with either the O γ of the active-site serine residue 195 or the N ϵ^2 of the imidazole group of histidine residue 57 to form either a B-O or B-N bond respectively. Boronic acids that are analogues of serine protease substrates form the former type of complex, those that are not analogues, the latter [35]. Natural, simple unsubstituted boric acid compounds (e.g., sodium borate) that contain a trigonal boron atom bind to serine proteases to form a reversible tetrahedral transition state analogue complex. For example, borate reversibly inactivates the serine protease, α -chymotrypsin, by accepting the free electron pair of the nitrogen atom on the imidazole group of the histidine residue at the active site [36]. At least five microbial subtilisin-type serine proteases bind to simple borates to form tetrahedral transition state analogues [37, 38].

It is reasonable to hypothesize that dietary boron helps regulate the normal inflammatory process by dampening the activity of leukocyte serine proteases and thereby reducing degradation of connective tissue structures and membrane constituents. Attempts are underway to measure the in vitro and in vivo activity of serine proteases during adjuvant-induced arthritis.

BORON AND HEMOSTASIS

Hemostasis, the physiologic coagulation of blood, is a specialized type of inflammatory response [39] and is affected by boron. There are at least three synthetic boroarginine peptides that are highly effective, slow-binding inhibitors of thrombin, the enzyme that converts fibrinogen into fibrin [26]. Their association rates with thrombin compare favorably with that for the macromolecular antithrombin III-heparin complex. In fact, the effective concentration of these boron-containing peptides is in the same effective range as hirudin, the anticoagulant isolated from the leech, *Hirudo medicinalis* [26]. There is indirect evidence that dietary boron (as boric acid) affects the coagulation system. Vitamin D adequate rats fed either boron low ($<0.2 \mu\text{g}$ [$0.02 \mu\text{mol}$]/g) or boron supplemented ($2.0 \mu\text{g}$ [$\sim 0.19 \mu\text{mol}$]/g) diets and inadvertently decreased amounts of vitamin K exhibited bleeding around the eyes and a high death rate. In this state of low blood-clotting ability, dietary boron increased the death rate further despite being provided in an amount only one-sixth of that typically present in commercial rodent chow ($\sim 13.0 \mu\text{g}$ [$1.2 \mu\text{mol}$]/g) [8]. Bleeding and death in both dietary groups ceased after restoration of dietary vitamin K [40]. Thus whereas boron may prevent pathologic coagulation, normal amounts of dietary boron apparently promote anticoagulation when vitamin K is limiting. In at least one published case of boric acid poisoning in humans [41], some symptoms of poisoning included capillary fragility and mild anemia. The patient recovered promptly when supplemented with vitamin K. Boron probably affects hemostasis by inhibiting serine proteases in the coagulation cascade, including coagulation Factors Xa, IXa, XIa, XIIa, and thrombin. Derivatives of vitamin K are required for the insertion of carboxyl groups in the precursors of prothrombin and Factors VII and IX. In the study described above with vitamin K deficient rats, the coagulation cascade was probably severely limited beyond Factors VII and IX, and prothrombin. It seems reasonable to speculate that reduced amounts of activated factor IX and thrombin, as well as the normal amounts of serine proteases higher in the coagulation cascade, were inhibited by boron to some degree. In the case of boric acid poisoning described above, the coagulation cascade of the patient was probably overinhibited by boron that may ordinarily act to prevent pathologic coagulation.

BORON AND EICOSANOIDS

The eicosanoids, a collective term for arachidonate and the prostaglandins, leukotrienes, and thromboxanes, are an important component of the inflammatory response. Essential fatty acids or their products are needed for the formation of the eicosanoids. Arachidonic acid and eicosapentaenoic acid are derived from n-6 or n-3 fatty acids, respectively. Oxygenation of arachidonic acid and eicosapentaenoic acid give rise to the prostaglandins, thromboxanes, and leukotrienes [42]. Lipoxygenase is required for the production of the leukotrienes, cyclooxygenase for the prostaglandins and thromboxanes.

Boron may play an important role in leukotriene metabolism at two different sites. First, studies with the sunflower indicate a direct inhibition of lipoxygenase. For example, in vitro studies demonstrate that boron inhibits (30% inhibition at $4 \mu\text{M}$ under standard reaction conditions) the lipoxygenase enzyme in the sunflower, a plant

very sensitive to either boron deficient or toxic media [43]. Studies of human leukocytes in culture with certain synthetic boron compounds, the 2'-deoxyribonucleoside cyanoboranes, reveal that these compounds inhibit leukocyte 5'-lipoxygenase activity and protect against free-radical formation generated in the Fenton reaction. The same compounds inhibited prostaglandin cyclooxygenase and 5'-lipoxygenase in cultured mouse macrophages [44].

Second, there is evidence that boron affects the metabolism of eicosanoids, especially the leukotrienes. The n-6 sulfidopeptide leukotrienes (LT) C_4 , LTD $_4$, and LTE $_4$ together constitute the biological activity ascribed to slow reacting substance of anaphylaxis (SRS-A). LTC $_4$ is converted to LTD $_4$ by GGT, and the bioconversion can be inhibited very strongly by serine-borate [45, 46]. An L-serine borate complex, 45 mM, reduced in vitro contractions induced by leukotriene LTC $_4$ in intralobar airways from human lung obtained after surgical resection [47]. Yet, L-serine borate unmasked contractile LTC $_4$ receptor mechanisms in isolated guinea-pig tracheal chains [48]. Because the leukotrienes are the major rate-limiting mediators of immune-inflammatory events in humans, boron may be in a key position to modulate the inflammatory process.

PRESENT RESEARCH FINDINGS: IMMUNE FUNCTION STUDY

Materials and Methods

Male, weanling, Sprague-Dawley rats (8/group) were fed either boron low (0.1 mg B/kg) or boron (as orthoboric acid [H $_3$ BO $_3$]) supplemented (2.0 mg B/kg) diets based on ground corn, high-protein casein, and corn oil and supplemented with adequate amounts of all vitamins (including vitamin D) and minerals considered essential for the growing rat [49]. They were made arthritic on d 41 of the experiment (postnatal d 63) by i.d. injection of *M. butyricum* [50] (Difco Laboratories, Detroit, MI) (0.15 mg in mineral oil) in the subplantar region of the right hindpaw. Neutrophil, monocyte, lymphocyte, and total white blood cell counts were determined immediately prior to injection of *M. butyricum* and subsequently on d 55 and 69. At the same times, wholeblood aliquots were dual-labeled with either CD4 (fluorescein isothiocyanate [FITC]-conjugated mouse antirat CD4) and CD8a (R-phycoerythrin [R-PE]-conjugated mouse antirat CD8a) monoclonal antibodies (Pharmingen, San Diego, CA) as markers of a T-lymphocyte cell subset (CD8a $^+$ /CD4 $^-$ -labeled); or CD11b/c (FITC-conjugated mouse antirat CD11b/c) and NKR-P1A (R-PE-conjugated mouse antirat NKR-P1A) monoclonal antibodies (Pharmingen) as markers of natural killer (NK) cells (NKR-P1A $^+$ /CD11b/c $^-$ -labeled). The percentage of labeled cells was determined by flow cytometry with NK cells isolated in the monocyte-lymphocyte gate and CD8a $^+$ /CD4 $^-$ cells in the lymphocyte gate. Left hind-paw (contralateral to injected foot) thickness was measured before induction and at several subsequent times using a gauge with dial readout (Dyer, Lancaster, PA).

Findings and Discussion

Boron effects on immune cells and tissue swelling. All rats grew at a normal rate (data not shown) and all exhibited signs of inflammation after injection with *M. butyricum*. Four of eight, but only one of seven rats fed the boron low and boron-supplemented diets, respectively, exhibited severe joint swelling at any time postin-

jection. After injection, supplemental dietary boron had a beneficial immunomodulatory effect by affecting the circulating concentrations of natural killer (NK) cells, the CD8a⁺/CD4⁻ cells, and neutrophils (Table I). For example, on d 14 after injection, rats fed the boron-supplemented diet compared to those fed the boron-low diet exhibited increased concentrations of NK and CD8a⁺/CD4⁻ cells. On d 28, but not d 14 postinjection, supplemental boron decreased neutrophil concentrations. Foot swelling was measured in the paw contralateral to the injected paw to determine the effects of boron on systemic responses to the injected antigen. Foot swelling remained relatively constant the first 10 d after injection regardless of dietary treatment, but then increased to a greater degree in rats fed the boron-low diet (Fig. 1).

Adjuvant arthritis is a well-recognized model of rheumatoid arthritis in humans because of the considerable overlap of clinical and pathologic features of each [50, 51]. As expected, injection of *M. butyricum* induced joint swelling [51] and leukocytosis with neutrophilia [50–52]. Whereas the effective dosage for treatment of joint pain by the Unani system may exceed amounts normally consumed in a typical diet, the effect of boron on arthritis in the present study cannot be considered pharmacologic, because the total amount of boron in the boron supplemented diet (2 mg B/kg) was only one-sixth of that typically present in commercial rodent chow (~13.0 mg B/kg) [8]. Furthermore, the large changes in circulating concentrations of NK cells, CD8a⁺/CD4⁻ cell, and neutrophils brought about by physiologic amounts of dietary boron can be considered beneficial because supplemental boron also attenuated the rate of hind foot swelling.

Boron and NK cell function. The finding that dietary boron increases circulating NK concentrations also provides support for the hypothesis that boron modulates the

TABLE I. Effect of Dietary Boron on Circulating Concentrations of Selected Immune Function Cells After Adjuvant-Induced Arthritis in Rats^a

Days ^b after <i>M. butyricum</i> injection	Dietary boron treatments		
	0.1 mg B/kg diet	2.0 mg B/kg diet	<i>P</i> values ^c
Natural killer cells ^d (million cells/mL whole blood)			
0 ^e	0.35 ± 0.13 ^f	0.45 ± 0.13	NS
14	0.22 ± 0.10	0.42 ± 0.13	0.008
28	0.17 ± 0.08	0.19 ± 0.13	NS
CD8a ⁺ /CD4 ⁻ -labeled, (million cells/mL whole blood)			
0	1.41 ± 0.38	1.94 ± 0.60	0.06
14	1.35 ± 0.47	2.02 ± 0.45	0.015
28	0.87 ± 0.32	0.98 ± 0.55	NS
Neutrophils (million cells/mL whole blood)			
0	1.31 ± 0.49	1.00 ± 0.17	NS
14	7.19 ± 5.11	3.45 ± 1.98	NS
28	2.06 ± 1.13	0.89 ± 0.20	0.02

^aAll rats fed same respective diets throughout study.

^bInjection in subplantar region of right hindpaw.

^ct-test.

^dNKR-P1A⁺/CD11b/c⁻-labeled.

^eDay 0 is d 41 of experiment (postnatal d 63).

^fMean ± standard deviation.

NS = not significant.

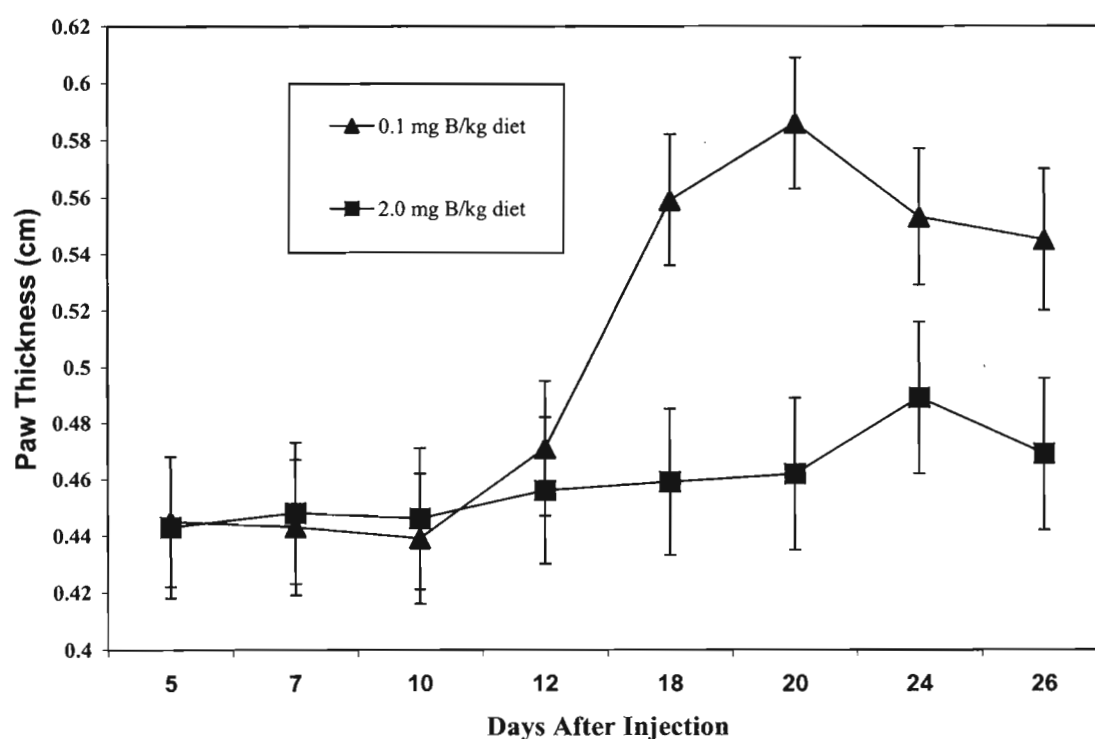


Fig. 1. Effect of dietary boron, length of recovery, and their interaction on paw swelling (Mean \pm SEM) after adjuvant-induced arthritis (i.d. injection with *M. butyricum* in the subplantar region of right hindpaw at age 63 d [d 41 of study]) in rats fed same respective diets throughout study. Paw thickness measurements were determined in the left hindpaw (metatarsal region). Analysis of variance: Boron, NS; Day, $P < 0.003$; Boron \times Day, $P < 0.01$.

inflammatory process, because it indicates that dietary boron serves as a possible regulator of the inflammatory response. Circulating NK cells, compared to other lymphocytes, divide rapidly [53] and comprise about 15% of blood lymphocytes. They are considered an important first line of defense against microbial infections, because their effector functions of cytolysis and cytokine (IFN- γ , TNF- α , and GM-CSF) secretion are not antigen specific and are activated immediately following contact with infected target cells [54]. The early production of IFN-gamma and possibly other cytokines is necessary to control certain bacterial, parasitic, and viral infections [55].

A monocyte-derived, suppressive signal effectively down-regulates the cytotoxic and proliferative activities of NK cells [56]. There is substantial evidence [57] that the signal requires intact monocyte NADPH oxidase activity. For example, monocytes recovered from patients with chronic granulomatous disease, a condition characterized by the absence of NADPH oxidase activity of phagocytes, do not inhibit NK cell function [57]. Also, the inhibition of NK cells requires cellular proximity to monocytes in vitro; the inhibition is prevented by catalase, an enzyme that degrades H_2O_2 into oxygen and water [57]. In summary, there are several lines of evidence that the cytotoxic and proliferative activities of NK cells are inhibited by H_2O_2 , a simple ROS that is released during respiratory burst activity of monocytes specifically and leukocytes in general (as described above). Therefore, it is reasonable to conclude that another beneficial effect of boron nutrition on ROS production is up-regulation of the cytotoxic and proliferative activities of NK cells.

NKR-P1 is a surface antigen expressed on all rat NK cells and only infrequently on a small subset of rodent T cells [58], a phenomenon that allows for highly specific labeling of NK with mouse antirat NKR-P1A monoclonal antibody. However, the mouse antirat CD8a monoclonal antibody from the OX-8 clone is used routinely to mark peripheral T-suppressor/cytotoxic cells [50], despite the fact that the OX-8 antibody also reacts with the hinge-like membrane-proximal domain of the alpha chain of the CD8 antigen expressed on thymocytes, intestinal intraepithelial lymphocytes, some activated T-helper cells, and, important for this discussion, most NK cells [59]. Thus the similar responses of NK cells and CD8a⁺/CD4⁻ cells to boron nutriture suggests that the response of the CD8a⁺/CD4⁻ cells reflects the expression of CD8a on most NK cells.

Conclusions

The findings from the latest study support the hypothesis that physiologic amounts of dietary boron reduce the risk for inflammatory disease by helping hold in check a system that is constantly poised to attack, a balance that permits elimination of pathogens but avoids autoimmunity. The hypothesis is based on the concept that boron serves as a suppressive signal that down-regulates enzymatic activities typically elevated during the normal inflammatory process.

SUMMARY

The literature and present research findings support the boron-inflammation hypothesis developed in this laboratory that dietary boron reduces the risk for inflammatory disease by serving as a suppressive signal that down-regulates enzymatic activities typically elevated during the normal inflammatory process. The findings from the present study provide the first *in vivo* evidence for an immunomodulatory effect of physiologic amounts of dietary boron. Specifically, there is now evidence that dietary boron helps control the normal inflammatory process by modulating the response of key immune cells to antigens. Vigorous attempts are underway in this laboratory to examine the effects of dietary boron on specific aspects of leukotriene, respiratory burst, and related cytokine metabolism and antibody production during the inflammatory response.

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